Table 1. INCORPORATION OF $^{32}P_i$ INTO ATP IN FOUR SEPARATE EXPERIMENTS

Composition of ghosts before sealing (mM)	Fraction of total counts incorporated into "energy rich" phosphate* in 15 min (per cent): "Na medium"†	"Na medium" + ouabain (5×10^{-5} g/ml)	"K medium"
ATP 1; P_i 5; Na 2; K 140; Mg 7; IAc 2	0·568	0·412	0·391
ATP 1; P_i 5; ADP 2; Na 4·8; K 132; Mg 6; IAc 2	0·667	0·571	0·535
ATP 1; P_i 5; Na 2; K 147; Mg 7; IAc 2; Iodoacetamide 5; NADH	0·344	0·261	0·228
ATP 1; P_i 5; Na 0; K 147; Mg 7; IAc 2; Iodoacetamide 5	0·302	0·235	0·205

* The ratio ATP counts/ADP counts was always about 3 : 1. [illegible] ADP is presumably labelled from ATP by adenylate kinase activity it is convenient to consider merely total "energy-rich" phosphate.

† The "Na medium" contained (mM): Na, 140; Mg, 7; Tris (pH 7·6), 5; IAc, 2. The "K medium" was similar but with potassium replacing 90 per cent of the sodium.

Table 2. INCORPORATION OF $^{32}P_i$ INTO ATP AND ADP

	Relative specific activities of nucleotides (Counts per mole nucleotide × 100) / Counts per mole P_i: "Na medium"*	"Na medium" + oligomycin (10 µg/ml)	"K medium"
ATP	1·89	1·57	1·37
ADP	0·89	0·83	0·72

* The "Na medium" was the same as in Table 1. The "K medium" was similar to the "Na medium" but with potassium replacing 90 per cent of the sodium. The composition of the ghosts before sealing was (mM): ATP 1; P_i 5; Na 0·2; K 150; Mg 7; IAc 2.

of AMP in the original experiment was intended to hold ATP at a low concentration through residual adenylate kinase activity[3]. It is possible, however, that ATP may be needed to "prime" the system[8], so in most of the later experiments the cells were lysed in solutions containing ATP as the only nucleotide. With 5 mM P_i and 1 mM ATP in the lysing solution, about one-third of the ATP trapped in the cells is split during the pre-incubation necessary for resealing; for the main part of the experiment the ratios P_i : ADP : ATP are therefore something like 15 : 1 : 2.

The results of four experiments are shown in Table 1. In these experiments cells were prepared with the compositions shown in column 1 and, after sealing[11] and washing, were incubated in sodium media with and without ouabain and in potassium media. After 15 min the cells were treated with trichloroacetic acid, unlabelled P_i, ATP and ADP were added to act as carriers, and the nucleotides were separated on 'Dowex' 1 columns. The amount of radioactivity found in each fraction was expressed as a percentage of the total radioactivity originally in the inorganic phosphate.

It is clear that with a high concentration of external sodium and zero external potassium there appears to be extra incorporation of P_i into ATP, and this extra incorporation is greatly diminished in the presence of ouabain. A separate experiment showed that ouabain did not affect the rate of incorporation of P_i when cells were suspended in a potassium medium.

A weakness of this kind of experiment is that because unlabelled nucleotides are added it is not possible to estimate the specific activities of the nucleotides in the cells. In the presence of external potassium, extra splitting of ATP through activation of the transport ATPase could lead to a lower concentration of ATP and therefore to fewer ATP counts even if the rate of formation of ATP were the same. With the very low concentration of internal sodium and the short period of incubation, the effect is likely to be small, and in any event it would not explain the ouabain result. Nevertheless, the difficulty can be overcome by carrying out the experiment on a scale sufficiently large to avoid the need for carriers, and estimating specific activities by direct comparison of counts and 259 mµ absorption. The results of such an experiment are summarized in Table 2 and show that $^{32}P_i$ incorporation really is greater in the sodium medium.

In this experiment [illegible] cardiac glycoside [illegible] (60 µg/mg protein) it partly inhibited the extra [illegible]poration associated with external sodium, and [illegible] is in agreement with the partial inhibition of [illegible] activity [illegible] sodium movements caused by similar concentrations of oligomycin[13-15].

Reversal [illegible] sodium pump leading to the formation of ATP is interesting because of [illegible] possible relevance to oxidative phosphorylation in mit[illegible] as well as for the light it may throw on the mechanism of the pump. The rate of the reversed reaction [illegible] these experiments was, very roughly, [illegible] ghosts/h, that is, about 10 per cent [illegible] of the [illegible] reaction in ghosts containing [illegible] ADP and P_i but with a [illegible] of [illegible] and about 2 per cent of the rate of the forward [illegible] in fresh red cells under physiological conditions.

We thank the Medical Research Council for [illegible] apparatus and the C[illegible] [illegible] Scientifique [illegible] for a personal [illegible] one of us (P. J. G.).

P. J. GARRAHAN
I. M. GLYNN

Physiological Laboratory,
University of Cambridge.

[1] Glynn, I. M., J. Physiol., 160, 18 P (1962).
[2] Sen, A. K., and Post, R. L., J. Biol. Chem., 239, 345 (1964).
[3] Whittam, R., and Ager, M. E., Biochem. J., 97, [illegible] (1965).
[4] Garrahan, P. J., and Glynn, I. M., J. Physiol., 185, 31 P (1966).
[5] Post, R. L., and Jolly, P. C., Biochim. Biophys. Acta, 25, 118 (1957).
[6] Skou, J. C., Biochim. Biophys. Acta, 42, 6 (1960).
[7] Fahn, S., Koval, G. J., and Albers, R. W., J. Biol. Chem., 241, 1882 (1966).
[8] Shaw, T. I. (personal communication).
[9] Garrahan, P. J., and Glynn, I. M., Nature, 207, 1098 (1965).
[10] Schrier, S. L., and Doak, L. S., J. Clin. Invest., 42, 756 (1963).
[11] Ronquist, G., and Agren, G., Nature, 209, 1090 (1966).
[12] Glynn, I. M., J. Physiol., 169, 452 (1963).
[13] Garrahan, P. J., and Glynn, I. M. (unpublished data).
[14] Hoffman, J. F., Tosteson, D. C., and Whittam, R., Nature, 185, 186 (1960).
[15] Järnefelt, J., Biochim. Biophys. Acta, 59, 643 (1962).
[16] Jöbsis, F. F., and Vreman, H. J., Biochim. Biophys. Acta, 73, 346 (1963).
[17] Van Groningen, H. E. M., and Slater, E. C., Biochim. Biophys. Acta, 73, 527 (1963).
[18] Whittam, R., Wheeler, K. P., and Blake, A., Nat[illegible]

PHARMACOLOGY

Determination of Nicotine and its Metabolite, Cotinine, in Urine by Gas Chromatography

A VARIETY of methods have been described [illegible] identification and determination of nicotine in bi[illegible] fluids[1-4]; however, they lack sensitivity, specific[illegible] [illegible] are tedious for routine analysis.

Gas chromatography has been used both qualit[illegible]ly and quantitatively to detect nicotine and related compounds in tobacco and in cigarette smoke[5]. In in[illegible]tions of the excretion of amphetamine, nicotine wa[illegible] to be eliminated in the urine of smokers[6,7] an[illegible] chromatographic method for the determination of [illegible] and its metabolite cotinine in biological fluids [illegible] scribed by McNiven et al.[8].

We have been using a simple quantitative gas chromatographic method for the determination of nicotine [illegible] cotinine in urine for about a year. In this [illegible] nicotine and cotinine showed single well resolve[illegible] metrical peaks, and no interfering substances [illegible] peaks were found in the hundreds of extracts [illegible] analysed from many subjects. Absolute recove[illegible] of these compounds and their standards from urine [illegible] follows: nicotine (90–95 per cent), chlorphen[illegible] hydrochloride (90–95 per cent), cotinine (85–90 per cent) and lignocaine hydrochloride (85–90 per cent). A [illegible] recovery of 95–100 per cent for nicotine and cotinine [illegible] obtained with respect to their internal standards [illegible] are added t[illegible]

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The sensitivity of this method makes it possible to use [illegible] all, the analysis takes about 6 h for sixteen samples.

The procedure [illegible] 5 ml. of urine [illegible] 1 ml. internal marker solution [illegible] 5 µg [illegible] ml. in [illegible] glass-stoppered centrifuge tubes. The solution [illegible] extracted with 3 × 2·5 ml. of freshly distilled 'Analar' diethyl ether, centrifuging to break any emulsion. [illegible] discarded and the urine [illegible] alkaline with 5 N sodium hydroxide (0·5 ml.). A further [illegible] ml. diethyl ether extracts were combined in a [illegible] tube, finely tapered at the base, and [illegible] to about 50 µl. at [illegible]; 3–5 µl. of this solution was injected on to the column using a 10 µl. Hamilton syringe.

A similar procedure [illegible] for cotinine except that there [illegible] extraction; (*b*) N ammonium hydroxide in place of the 5 N sodium hydroxide; (*c*) freshly distilled [illegible] dichloride as the extracting solvent; [illegible] hydrochloride, 5 µg base/ml. in distilled water [illegible] standard.

A [illegible] with a flame ionization detector [illegible] 5 mV Leeds and Northrup Speedomax [illegible], model S, were used. The [illegible] tubing 1/8 in. [illegible] packed with 80–100 [illegible] per cent w/w potassium hydroxide and [illegible] 20 *M*. The operating conditions [illegible] hydrogen pressure, 15 [illegible] air pressure, 25 [illegible] nitrogen flow rate, 35 [illegible] split ratio 1 : 2. Injection block temperature about 250° C; oven temperature 175° C (nicotine) and 200° C (cotinine). The column was [illegible] with 2 × 5 µl. of [illegible] after equilibrating for 24 h at the operating conditions.

The concentrations of nicotine and cotinine were obtained by calculating the ratio of peak height of the compound to that of the internal marker and relating this to a previously constructed calibration curve of the compound in urine.

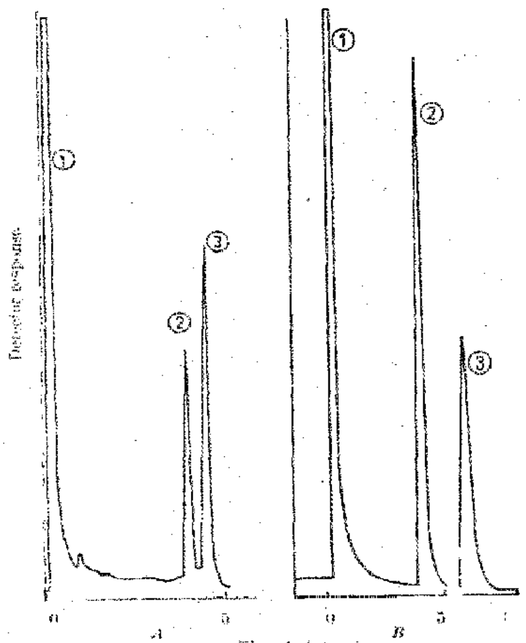

Fig. 1. *A*, Chromatogram [illegible] from urine [illegible] *B*, [illegible]

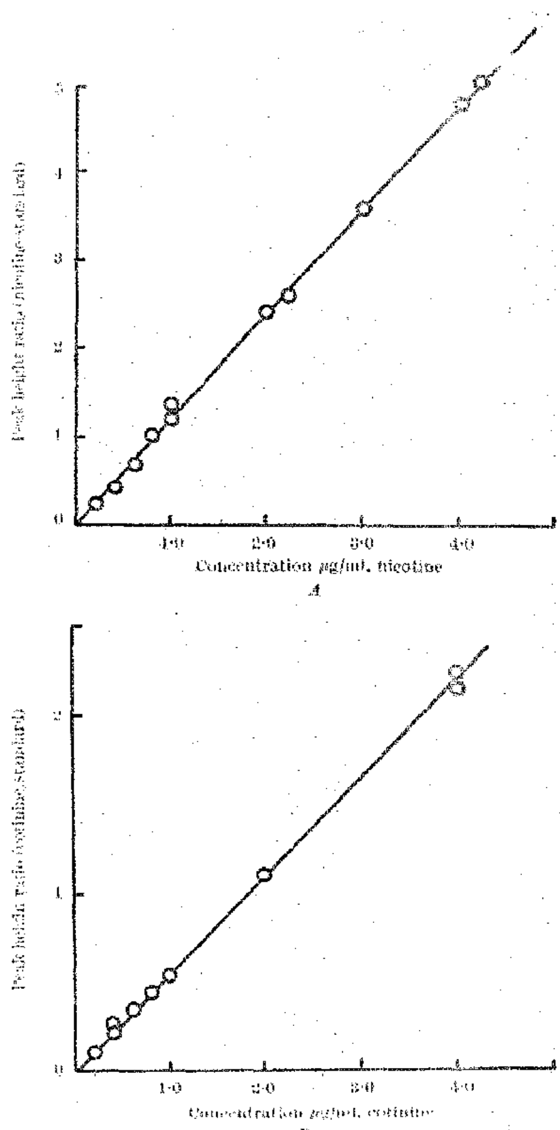

Fig. 2. *A*, Calibration curve for nicotine. *B*, Calibration curve for cotinine.

Figs. 1*A* and 1*B* show chromatograms of nicotine and cotinine with their internal standards. No deterioration of the compounds in urine occurred when [illegible] stored for 2 weeks at 4° C, and the extracts [illegible] trates were stable for 24 [illegible]

Figs. 2*A* and 2*B* show calibration curves for nicotine and cotinine. A consistent linear detector response [illegible] for both compounds over the range 0·2 [illegible] ml. urine. Sixteen analyses of a urine [illegible] 1·0 µg of nicotine base/ml. gave a standard deviation of [illegible] µg/ml.; similarly for cotinine [illegible] standard deviation of 0·05 µg/ml. [illegible]

[illegible] the procedure described for [illegible] it was found that the rate of [illegible] in urine was influenced by the pH of the urine [illegible] refs. 7, 8), and by the [illegible] the rate of flow, the [illegible] This was also found to apply to the [illegible] of cotinine. Subjects who simultaneously [illegible] two cigarettes [illegible] abstaining from [illegible] period of 24 h excreted 0·25–0·60 [illegible]

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[illegible] 0.5 mg nicotine (in 24 h) in the normal [illegible] 0.3–0.6 mg nicotine (in 22 h) and 0.6–0.9 mg nicotine (in 8 h) when the urine was made acidic after oral administration of ammonium chloride, and 0.5–0.9 mg nicotine (in 20 h) and less than 0.05 mg nicotine (in 3 h) when the urine was made alkaline after oral administration of sodium bicarbonate.

Absorption of nicotine from the lungs and excretion in the urine were found to be extremely rapid, as the maximal rate of excretion was in the period 0–1 min after smoking. The rate of nicotine excretion under these conditions fell very rapidly to less than 0·1 μg/min by 3 min after smoking the two cigarettes. The elimination of nicotine did not appear to follow first order kinetics and this is being further investigated. The peak level of nicotine excretion was found to be at about 2 h after smoking. The subjects were heavy smokers (twenty cigarettes a day) and intermittent smokers (between two and five cigarettes a week), and for the purpose of the experiments inhaled the smoke from the non-filter cigarettes used.

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A. H. Beckett
E. J. Triggs

School of Pharmacy,
Chelsea College of Science and Technology,
Manresa Road,
London, S.W.3.

Jakschewitz, B., *Hoppe-Seyler's Z. Physiol. Chem.*, **292**, 91 (1953).
Fabre, R., *et al.*, *Ann. Pharm. Franc.*, **10**, 577 (1952).
Ferebee, H., *et al.*, *Arch. Mal. Prof.*, **24**, 432 (1963).
[illegible] and Kelsey, F. E., *Fed. Proc.*, **9**, 271 (1950).
Pelikan, E. W., and Pappas, N. A., *Intern. Rec. of Chem.*, **10**, 79 (1965).
Schlittler, E., and Domanski, G., *Prog. in Chem. Toxicol.*, **2**, 93 (Academic Press Inc., New York, 1965).
Scott, A. B., Rowland, R., and Triggs, E. J., *Nature*, **207**, 200 (1965).
McKennis, H., *et al.*, *Nature*, **205**, 700 (1965).
Haag, H. B., and Larson, P. S., *J. Pharm. Exp. Therap.*, **76**, 235 (1942).

HAEMATOLOGY

Rapid Induction of *in vivo* Fibrinolysis with D-Gluconyl-L-tyrosine

RECENTLY I reported[1] lysis of thrombi in the veins and arteries of animals with sodium gluconate and a dialysable constituent of fibrin extract or whole blood. The ultraviolet absorption spectrum of fibrin extract displayed a maximum at 275 mμ and a minimum at 247 mμ. These values happen to coincide closely with those recorded for tyrosine. This finding suggested that L-tyrosine might be successfully substituted for fibrin extract and, if this was so, then a peptide formed from the amino-acid and gluconic acid would also be fibrinolytic.

Clots were induced in the jugular veins of dogs as previously described[1], and infusions administered locally without the admission of blood. A mixture of 100 mg of L-tyrosine and 1 g of sodium gluconate lysed such thrombi, whereas neutralized gluconate or L-tyrosine with sodium gluconate were ineffective. D-Gluconyl-L-tyrosine was prepared by the method of Iacobellis[2]. One hundred milligrams in 1 ml. of distilled water injected into a vein within 15 min the [illegible] of blood in excised jugular veins of dogs weighing 2–3 kg.

The experimental fibrin clots *in vitro*. It may be significant as the prosthetic group of a larger molecule, presumably a protein.

PAUL H. KOPPER

Department of Biology,
Salisbury University,
Salisbury, Maryland.

Kopper, P. H., *Nature*, **211**, 43 (1966).
Iacobellis, S., *Arch. Biochem. Biophys.*, **59**, 130 (1955).

IMMUNOLOGY

Complement-fixing Antibodies in Monkeys bearing Tumours induced by Rous Sarcoma Virus

Rous sarcoma virus (RSV) produces tumours in rabbits, monkeys, sheep and dogs[1–7]. RSV tumours in hamsters, guinea-pigs and rats contain complement-fixing (CF) antigens which elicit antibodies to homologous virus-free RSV-induced hamster tumour antigens, virus-containing RSV-induced chicken tumours, and antigens induced by the growth of several strains of avian leukosis viruses in chick embryo fibroblast (CEF) tissue cultures[7,8].

Recently, using methods previously described[7,8], we observed the presence of antibodies specific for avian leukosis in the serum of a rhesus monkey (family supplied by F. J. Rauscher) which developed tumours after inoculation at birth with the Schmidt-Ruppin (SR) strain of RSV. This monkey, the offspring of a healthy young mother, was bled for a serum sample when less than 24 h old and then inoculated subcutaneously over both the right and left lower back with 0·5 ml. of SR-RSV prepared from chicken wing web tumours[8].

A small subcutaneous tumour was first noted at the site of injection on the right side 21 days after inoculation of the virus. Several subcutaneous tumours afterwards developed and grew progressively at both inoculation sites. Two tumours which developed on the left side were excised 7 days after they were first noted. Attempts to establish cells from these excised tumours in tissue culture were unsuccessful. Tumours recurred rapidly at the site of the excised tumours and were readily detectable 7 days after excision. In several attempts to establish tumour cells from the subcutaneous nodules, explantations of the tumours have been performed 30 days after their original inoculation. Tumours successfully grown in hamsters and re-excised through six subpassages were established and carried by Dr. H. Graham. In these experiments we noticed at the excision sites the formation of firm subcutaneous nodules which were indistinguishable from tumour tissue by palpation, but these regressed rapidly and left no gross evidence of tumours 6 weeks later.

Five to six weeks after the onset of tumours, CF antibodies appeared in the monkey serum which reacted with SR strain hamster tumour antigens and the antigens found in chicken tumours induced by both SR and Bryan strains of RSV but not with the virus-induced hamster tumour antigens of ST40 virus, polyoma virus or adenovirus types 7 and 12. The monkey sera with the highest antibody titres against RSV hamster and chicken tumour antigens also reacted specifically with antigens present in CEF cultures of both resistance-inducing factor (RIF) and avian erythroblastosis virus (AEV), but all sera were negative when tested against cell pack preparations of tumourless CEF cultures used at the same dilutions as the avian leukosis antigens. The progress of specific CF antibody formation is illustrated in Table 1 which shows the reaction of four consecutive serial tests tested against specific antigens indicated. Antibody titre generally increased during the period of tumour development but fell to undetectable levels within 2 weeks of tumour excision and subsequent regression of the tumours. The sera with highest antibody titres were positive at 1 : 10 dilution when tested 5 weeks after inoculating antibodies against both SR and Bryan strains of RSV. Ten months after excision, tumours did not recur, and serum taken at that time did not react in CF tests with RSV antigens.

Despite the development of CF antibodies, we were unable to demonstrate CF antigens in a 20 per cent extract of tumour tissue excised from this monkey when it was tested at 1 : 2 dilution versus three of the highest titred homologous monkey sera or against standard

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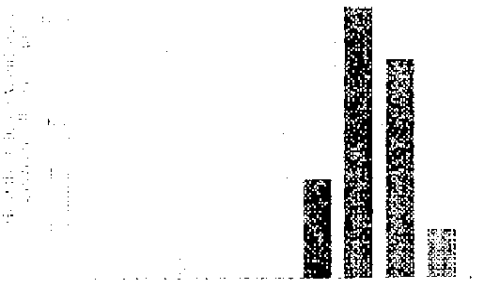
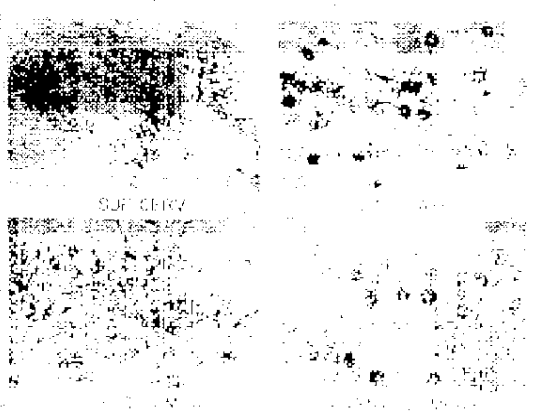
Morphological Correlate to a Cholinergic Sympathetic Function





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Physiological Disposition and Fate of Nicotine labelled with Carbon-14 in Mice

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Fig. 1. Aut[illegible] in a mouse 15 min. after intra[illegible] of nicotine labelled with carbon-14. [illegible] concentration [illegible]

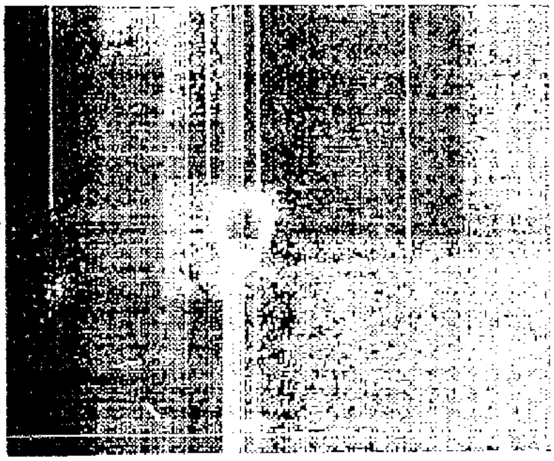

Fig. 2. [illegible] the border between the medulla and the cortex

30 min. and 1, 2 and 4 hr. after the injection by immersing them into a mixture of acetone and carbon dioxide ice, after which autoradiography was performed[7].

Judging from the whole-body autoradiograms it is obvious that a short time (1-2 min.) after intramuscular injection a considerable part of the radioactive nicotine has been absorbed from the site of [illegible]ction. The most remarkable finding in these [illegible] is the high brain concentration (Fig. 1). The [illegible] in the brain and spinal cord is consider[illegible] than in other tissues, and throughout the entire [illegible] of [illegible] (up to 4 hr. after [illegible] a high content of radio[illegible] 30 min. after inj[illegible] a concentration of radio[illegible] gastric mucosa and radio[illegible] in the lumen of the stomach. [illegible] of the nasal cavity [illegible] of radioactivity. In pregnant [illegible] found in the foetuses, indicating that nicotine [illegible] placental barrier.

The main excretory [illegible] is by the urine. A few minutes [illegible] the injection radioactivity can be observed in the renal hilus and in the urinary [illegible]. Another [illegible] for nicotine [illegible] the stomach.

Another [illegible] finding is the high concentration of nicotine in the adrenal medulla [illegible] after injection, and [illegible] of observation. [illegible] radioactivity [illegible] Experiments are in progress [illegible] nicotine in the adrenal medulla and [illegible]

Metabolism investigations show that the nicotine [illegible] rapidly [illegible] in the [illegible] brain and the foetuses, however, only [illegible] was observed (presumably cotinine). The autoradiographic picture demonstrates thus both nicotine and its metabolites. Experiments are in progress in order to follow the metabolic rate of nicotine in different organs and also to identify the metabolic compounds.

This investigation was supported by a grant from the Swedish Tobacco Co.

C. G. SCHMITERLÖW
E. HANSSON

Department of Pharmacology,
Kungl. Veterinärhögskolan,
Stockholm.

[illegible] *Exp. Path.*, 98, 370 (1923).
[illegible] E., *Biochem. Z.*, 318, 591 (1948).
[illegible] A., *Ann. Chem.*, 562, 60 (1950).
[illegible] Y., Yoshimoto, S., and Kumura, I. [illegible] 26 (1955).
[illegible] F. E., and Geiling, E. M. K., *J. Pharm. Exp.* [illegible] (1951).
[illegible] Wada, E., Bowman, E. R., and Turnbull, L. B. [illegible] (1961).
[illegible] *Radiol.*, Supp. 118 (1954).

HISTOCHEMISTRY

Histochemical Adenosine Triphosphatase Activity in Dark and Light Cells of Collecting Tubules in the Mammalian Kidney

In the kidneys of various species, the collecting tubules are composed of two types of cells. These were [illegible] by Heidenhain[1], and described [illegible] light and dark (intercalated) cells. The name given to these cells alludes to the fact that the cytoplasm of the dark cells stains with various commonly used dyes more intensely than that of the light cells. The dark (or intercalated) cells reveal also strong metachromasia[2]. Electron micrographs show that the dense cytoplasm of the dark cells contains more RNA granules than the cytoplasm of the light cells. Mitochondria are more numerous in the dark cells and are localized not only in the basal but also in the apical portion of the cytoplasm[3]. In contrast, electron-dense gra[illegible] are more numerous in the light cells than in [illegible] cells. [illegible] are composed of phospho[illegible] and [illegible] contain one or several cryst[illegible] lattice[4].

[illegible] reactions which localize enzymatic activity [illegible] show a marked difference in staining [illegible] This difference has been [illegible] previously [illegible] and tri- and diphosphopy[illegible] diaphorase in the rat kidney[5]. [illegible] in the staining reaction [illegible] triphosphatase is recorded.

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